In the event this paper is not being timely filed, the applicants respectfully petition for an appropriate extension of time. Any fees for such an extension together with any additional fees may be charged to Counsel's Deposit Account 01-2300.

Respectfully submitted,

Arent Fox Kintner Plotkin & Kahn

Robert K. Carpenter

Robert K. Carpenter
Attorney for Applicants
Reg. No. 34,794

1050 Connecticut Ave. NW
Suite 600
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810

RKC/lcw

Enclosures: Protein Alignment
Abstracts from 8 references

ANHANG:

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chGDF5
APLATRQGKRPSKNLKPRCSRKALHVNFKDMGWDDWIIAPLEYEAYHCEGLCEFPLRSHL
huGDF5
APLATRQGKRPSKNLKARCSRKALHVNFKDMGWDDWIIAPLEYEAFHCEGLCEFPLRSHL
mGDF5
APLANRQGKRPSKNLKARCSRKALHVNFKDMGWDDWIIAPLEYEAFHCEGLCEFPLRSHL

****.**********.*****************************:**************

chGDF5
EPTNHAVIQTLMNSMDPESTPPTCCVPTRLSPISILFIDSANNVVYKQYEDMVVESCGCR
huGDF5
EPTNHAVIQTLMNSMDPESTPPTCCVPTRLSPISILFIDSANNVVYKQYEDMVVESCGCR
mGDF5
EPTNHAVIQTLMNSMDPESTPPTCCVPTRLSPISILFIDSANNVVYKQYEDMVVESCGCR

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United States Patent
Lucas , Ê et al.

4,931,548

June 5, 1990

Heterodimer form of transforming growth factor-beta

Abstract

A polypeptide transforming growth factor found in porcine platelets, having activity in the TGF-.beta. assay and a molecular weight of about 25 kDa. The factor is a heterodimer, one chain of which has an N-terminal sequence very different from human platelet TGF-.beta., and the other chain of which has an N-terminal sequence identical to that of human platelet TGF-.beta.. The factor is purified using gel filtration and reverse phase HPLC.

Inventors: Lucas; Roger C. (Blaine, MN); Weatherbee; James A. (St. Anthony, MN); Tsang; Monica L.-S. (St. Anthony, MN)
Assignee: Techne Corporation (Minneapolis, MN)

Appl. No.: 008808
Filed: January 30, 1987

United States Patent
Ogawa , Ê et al.

5,413,989

*May 9, 1995

Method and activin compositions for inducing bone growth

Abstract

Activin is administered systemically and locally to induce the growth of mature bone. Activin enhances the level of bone formation and the quality of the bone formed when administered locally with BMP or

bone marrow. Administration of activin by subcutaneous route promotes systemic increase in the bone mass.

Inventors: Ogawa; Yasushi (Pacifica, CA); Schmidt; David K. (Santa Cruz, CA); Armstrong; Rosa (Palo Alto, CA); Nathan; Ranga (Newark, CA); Thompson; Andrea Y. (Mountain View, CA); Seyedin; Saeid M. (Saratoga, CA)

Assignee: Celtrix Pharmaceuticals, Inc. (Santa Clara, CA)

United States Patent 5,462,925

Ogawa , et al.

October 31, 1995

Transforming growth factor .beta.2,3

Abstract

A heterodimeric form of TGF-.beta. is described. This 25 KD molecule is active in an in vitro assay of inhibition of epithelial cell growth. The protein may be isolated from bone. When reduced, the protein elutes in two peaks by RP-HPLC. In immunoblots, the reduced protein from the earlier eluting peak reacts predominately with antibodies directed against TGF-.beta.3, while reduced protein from the later eluting peak reacts predominately with antibodies directed against TGF-.beta.2. The N-terminal amino acid sequence and immunoreactivity of the native protein are consistent with a heterodimer of TGF-.beta.2 and TGF-.beta.3.

Inventors: Ogawa; Yasushi (Pacifica, CA); Schmidt; David (Santa Cruz, CA); Dasch; James (Palo Alto, CA)

Assignee: Celtrix Pharmaceuticals, Inc. (Santa Clara, CA)

Appl. No.: 979441

Filed: November 20, 1992

United States Patent 5,482,851

Derynck , et al.

January 9, 1996

Nucleic acid encoding TGF-.beta. and its uses

Abstract

Nucleic acid encoding TGF-.beta. has been isolated and cloned into vectors which are replicated in bacteria and expressed in eukaryotic cells. TGF.beta. is recovered from transformed cultures for use in known therapeutic modalities. Nucleic acid encoding TFG-.beta. is useful in diagnosis and identification of TGF-.beta. clones.

Inventors: Derynck; Rik M. A. (So. San Francisco, CA); Goeddel; David V. (Hillsborough, CA)

Assignee: Genentech, Inc. (S. San Francisco, CA)

Appl. No.: 147364

Filed: November 5, 1993

J Biol Chem 1992 Feb 5;267(4):2325-8

Purification and characterization of transforming growth factor-beta 2.3 and -beta 1.2 heterodimers from bovine bone.

Ogawa Y, Schmidt DK, Dasch JR, Chang RJ, Glaser CB

Celtrix Laboratories, Palo Alto, California 94303.

A unique form of transforming growth factor-beta (TGF-beta), TGF-beta 2.3 heterodimer, has been purified from bovine bone extract. TGF-beta 2.3 migrated as a single 25-kDa band by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, whereas under reducing conditions it migrated as a 12.5 kDa band. The TGF-beta 2.3 reacted positively with anti-TGF-beta 2 and anti-TGF-beta 3 antibodies on immunoblots. Equal levels of TGF-beta 2 and TGF-beta 3 sequences were detected by N-terminal sequencing. TGF-beta 2.3 eluted as a single sharp peak by reverse-phase high performance liquid chromatography. However, prior reduction of the protein with dithiothreitol resulted in the protein eluting in two peaks, one containing predominantly TGF-beta 3 and the other containing predominantly TGF-beta 2. TGF-beta 2.3 inhibited proliferation of mink lung epithelial cells and promoted the formation of colonies of normal rat kidney fibroblasts in culture with specific biological activity similar to those of TGF-beta 1 and TGF-beta 2. These results demonstrate that the protein is TGF-beta 2.3 heterodimer, consisting of one polypeptide chain each of TGF-beta 2 and TGF-beta 3 linked by one or more disulfide bonds. In addition, TGF-beta 1.2 heterodimer, previously found only in porcine platelets, has also been purified from bovine bone extract.

J Biol Chem 1988 Aug 5;263(22):10783-9

Heterodimeric transforming growth factor beta. Biological properties and interaction with three types of cell surface receptors.

Cheifetz S, Bassols A, Stanley K, Ohta M, Greenberger J, Massague J

Department of Biochemistry, University of Massachusetts Medical School, Worcester 01655.

Type beta transforming growth factors (TGF) are disulfide-linked homo- and heterodimers of two related polypeptide chains, beta 1 and beta 2. The homodimers TGF-beta 1 and TGF-beta 2 are widely distributed, but the heterodimer TGF-beta 1.2 has been found only in porcine platelets (Cheifetz, S., Weatherbee, J.A., Tsang, M.L.-S., Anderson, J.K., Mole, J.E., Lucas, R., and Massague, J. (1987) Cell 48, 409-415). Here we characterize the receptor binding and biological properties of TGF-beta 1.2 and compare them with those of TGF-beta 1 and TGF-beta 2. Three types of cell surface receptors previously identified by affinity labeling with 125I-TGF-beta 1 are available for binding to TGF-beta 1.2. These three types of receptors are detected as 65-kDa (type I), 85-95-kDa (type II), and 250-350-kDa (type III) affinity-labeled receptor complexes on electrophoresis gels. They co-exist in many cell types, have high affinity for TGF-beta 1, and varying degrees of affinity for TGF-beta 2. Of the 11 cell lines screened in the present study none showed evidence for additional receptor types that would bind TGF-beta 2 but not TGF-beta 1. In receptor competition studies, TGF-beta 1, TGF-beta 1.2, and TGF-beta 2 competed for binding to type I and type II receptors with a relative order of potencies of 16:5:1 and 12:3:1, respectively, whereas all three forms of TGF-beta were equipotent as ligands for the type III receptors. The three forms of TGF-beta were equally potent at stimulating the biosynthesis of extracellular sulfated

proteoglycan in BRL-3A rat liver epithelial cells, a response that presumably involves the type III receptor present in these cells. In contrast, the ability of the three ligands to inhibit the growth of B6SUt-A multipotential hematopoietic progenitor cells which display only type I receptors decreased in the order TGF-beta 1, TGF-beta 1.2, and TGF-beta 2 with a relative potency of 100:30:1. The results indicate that the presence of one beta 1 chain in TGF-beta 1.2 increases (with respect to TGF-beta 2) the biological potency and binding affinity toward receptor types I and II, but the presence of a second beta 1 chain in the dimer is required for full potency.

Mol Endocrinol 1991 Jan;5(1):149-55

Bone-inducing activity of mature BMP-2b produced from a hybrid BMP-2a/2b precursor.

Hammonds RG Jr, Schwall R, Dudley A, Berkemeier L, Lai C, Lee J, Cunningham N, Reddi AH, Wood WI, Mason AJ

Department of Developmental Biology, Genentech, Inc., South San Francisco, California 94080.

The human osteoinductive proteins BMP-2a and BMP-2b have been cloned and expressed in mammalian cells. In order to improve expression levels we examined the role of the proregion in assembly and export. Use of the BMP-2a proregion combined with the mature region of BMP-2b leads to dramatically improved expression of mature BMP-2b. Mature BMP-2b has been purified to near homogeneity from the BMP-2a/2b hybrid, and its structural properties and biological activity determined. Recombinant mature BMP-2b homodimer elicits bone formation in vivo.

J Biol Chem 1992 Oct 5;267(28):20352-62

Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro.

Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, White KH, Coughlin JE, Tucker MM, Pang RH, et al

Creative BioMolecules Inc., Hopkinton, Massachusetts 01748.

We reported previously that a 32-36-kDa osteogenic protein purified from bovine bone matrix is composed of dimers of two members of the transforming growth factor (TGF)-beta superfamily: the bovine equivalent of human osteogenic protein-1 (OP-1) and bone morphogenetic protein-2a, BMP-2a (BMP-2). In the present study, we produced the recombinant human OP-1 (hOP-1) in mammalian cells as a processed mature disulfide-linked homodimer with an apparent molecular weight of 36,000. Examination of hOP-1 in the rat subcutaneous bone induction model demonstrated that hOP-1 was capable of inducing new bone formation with a specific activity comparable with that exhibited by highly purified bovine osteogenic protein preparations. The half-maximal bone-inducing activity of hOP-1 in combination with a rat collagen matrix preparation was 50-100 ng/25 mg of matrix as determined by the calcium content of day 12 implants. Evaluation of hOP-1 effects on cell growth and collagen synthesis in rat osteoblast-enriched bone cell cultures showed that both cell proliferation and collagen synthesis were stimulated in a dose-dependent manner and increased 3-fold in response to 40 ng of hOP-1/ml. Examination of the expression of markers characteristic of the osteoblast phenotype showed that hOP-1 specifically stimulated the induction of alkaline phosphatase (4-fold increase at 40 ng of hOP-1/ml), parathyroid hormone-mediated intracellular cAMP production (4-fold increase at 40 ng of hOP-1/ml), and osteocalcin synthesis (5-fold increase at 25 ng of hOP-1/ml). In long-term (11-17 day) cultures of osteoblasts in the presence of beta-glycerophosphate and L(+)-ascorbate, hOP-1 markedly increased the rate of mineralization as measured by the number of mineral nodules per well (20-fold increase at 20 ng of hOP-1/ml). Direct comparison of TGF-beta 1 and hOP-1 in these bone cell cultures indicated that, although both hOP-1 and TGF-beta 1 promoted cell proliferation and collagen synthesis, only hOP-1 was effective in specifically stimulating markers of the osteoblast phenotype.